Amphipod *Hyalella azteca* Sediment Toxicity Test

1.0 OBJECTIVE

Many anthropogenic contaminants in aquatic systems become bound to particles and may subsequently accumulate in sediments. Sediment toxicity to freshwater epibenthic amphipods can be assessed using this Standard Operating Procedure (SOP). Observed effects may be related to the presence of contaminants or to naturally occurring factors. To correctly interpret toxicity results, a number of parameters should be analyzed, including sediment chemistry, grain size, and TOC, as well as those specified below (pH, dissolved oxygen, hardness, alkalinity, temperature, ammonia and conductivity.

In this procedure, sediment collected from field stations is divided into randomly numbered replicate test containers in the laboratory and covered with dilution water (US EPA 2000). Ten amphipods are placed into each replicate container and allowed to interact with the test sediments. After a 10-day exposure, the sediment is sieved to recover the amphipods, and live animals are counted to determine the percentage that survived the exposure. Animals from each replicate are then dried and weighed. Sediment toxicity is characterized by the mean percent survival and growth (± standard deviation) for each sediment sample. This can be compared to the survival and growth observed in sediment from the amphipod collection site (home sediment), or in sediment from reference sites presumed to have similar natural characteristics but low contaminant concentrations (Kemble *et al.* 1994).

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (SOP).

2.1 Culture

- Large, clean culture tray to hold and acclimate *Hyalella*
- Air stones, tubing, and clean air system
- Granite Canyon well water (23± 1°C) for renewals
- YCT for feeding, purchased from Aquatic Biosystems (Fort Collins, CO)
- *Hyalella azteca*, 7-14 days old, supplied by Chesapeake Cultures (Hayes, VA)

2.2 Test Initiation

- Environmental chamber (23± 1°C, ambient laboratory illumination for 16 hours/day)
- 1-liter polycarbonate Imhoff settling cones (8 per sample), and rack for cones
- Clean air supply and air manifolds, with air tubing and glass pasteur pipettes
- Silicone stoppers (size 4) (1 per Imhoff cone)

• 250-ml clean plastic beakers (15) with covers for reference toxicant test
• 1000-ml clean plastic volumetric flask, 10-ml pipettor and glass pipettes for reference toxicant dilutions
• Cadmium chloride stock solution (100 mg/L)
• Mortar and pestle for grinding reference dirt
• Clean polypropylene spoons to scoop sediment into containers (one per sediment sample)
• Clean 250-ml crystallizing dishes to sort amphipods
• Clean glass tubes with squeeze bulbs for sorting amphipods
• Hand counters
• Randomization sheet to arrange and identify test containers
• Data sheets
• Gloves and appropriate safety gear (see MPSL lab safety manual)
• Sample vials for reference toxicant analysis (new polyethylene 30 ml, acid washed)
• Graduated pipettes (10 ml) and hand pipette pump for feeding YCT

2.3 Water Quality

• Meters, probes, spectrophotometer, digital titrator and standards for measuring pH, dissolved oxygen, hardness, alkalinity, ammonia, and conductivity
• Thermometers (glass spirit thermometer and continuously recording thermometer)
• Graduated pipettes (10 ml) and hand pipette pump for water quality sampling
• Water quality vials (30 ml glass)
• Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 Dilution Water

In every step of this procedure, use Granite Canyon well water.

2.5 Test Maintenance

• Thermometer (glass spirit) for daily temperature check
• Graduated pipettes (10 ml) and hand pipette pump for feeding YCT

2.6 Test Termination

• Toxic 400-µm screens to sieve amphipods at test termination
• Toxic glass tubes with squeeze bulbs and screens for collecting amphipods
• Tared weigh boats, small sieve, tweezers, drying oven and balance for drying and weighing individuals
• Data sheets and clipboards
• Pens (and towel to dry hands for writing)
• Gloves and appropriate safety gear (see MPSL lab safety manual)

3.0 EXPERIMENTAL DESIGN

Sediment toxicity tests can be used as screening tools or as part of more comprehensive studies to assess sediment quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of eight replicate test containers for each sediment sample. Containers are arranged randomly, and each receives ten amphipods. The quality of test amphipods and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and home (collection site) sediment (negative controls). Testing of reference sites is recommended to demonstrate the suitability of test sediments in the absence of toxic contaminant concentrations. Test conditions of temperature and photoperiod are controlled as indicated below, and pH, NH_3, salinity, dissolved oxygen, alkalinity, hardness and temperature are measured at the beginning and end of the exposure.

4.0 PREPARATION OF SEDIMENTS FOR TESTING

Label test cones as indicated on the randomization sheet generated for the test. Remove test sediments from refrigerated storage and place samples on the lab bench prepared for distributing sediment into test beakers. Carry a small number of samples at a time to avoid injury and possible loss of samples to breakage. Minimize sample exposure to sunlight (never leave samples in direct sunlight), and schedule loading times to avoid prolonged sample exposure to temperatures above 23°C. Do not sieve, freeze, or allow test sediments to dry prior to testing. Remove large objects such as sticks or clams with forceps, and note their presence on the data sheet.

Using a separate clean polypropylene spoon for each sample, re-homogenize (stir) the sediment in the sample jar to thoroughly mix overlying water back into the sediment. Spoon 15 ml of sediment into each of the eight test cones. Leave the spoon in the sample jar so it won't be used for other samples. Add well water to the 1-l level carefully so as not to disturb the sediment. Arrange the test beakers in numerical order on racks in the constant temperature room.

5.0 CONTROLS

5.1 Dirt Controls (Negative Controls)

Hydrate enough finely ground *Hyalella* control dirt for 8 replicates. Alternate adding water and dirt until the desired volume is attained. Using a clean polypropylene spoon, distribute the wet dirt into eight test cones. Carefully add well water to the 1-l level.

5.2 Reference Toxicant Tests (Positive Controls)

For cultured organisms, conduct a concurrent reference toxicant at least monthly. The reference toxicant test is a 96-hour exposure using a water-only dilution series of cadmium chloride, and provides data on the relative sensitivity of cultured organisms.

Reagent grade cadmium chloride ($CdCl_2$) should be used as the reference toxicant for *Hyalella* tests, unless another toxicant is specified by the Regional Water Quality Control Board or other appropriate regulatory agency. Prepare a 100 mg/l Cd stock solution by adding 0.1630 g of reagent grade $CdCl_2$ to a final volume of one liter of distilled water in a plastic volumetric flask. Cap tightly and mix thoroughly. Sample and log the reference toxicant stock solution at the beginning of the test for chemical verification of the cadmium concentration. Acidify samples for analysis in metals-clean sample vials with 300 µl of 14N double-distilled nitric acid per 30 ml of sample.

Reference toxicant solutions should be three to five replicates of 0 (control), 5, 10, 20 and 40 µg Cd/liter. Other concentrations may be added between these if greater precision is desired for quality control chart purposes. Prepare 1 liter of each concentration by adding 0, 50, 100, 200 and 400 µl of stock solution, respectively, to a 1-liter plastic volumetric flask and fill with culture water. Aliquot each concentration to randomly numbered test containers as indicated on the random number sheet. Start with the control solutions and progress to the highest concentration to minimize contamination. Place the reference toxicant test containers in the constant temperature room, cover, and equilibrate. Aeration is not necessary.

All tests (sample and reference toxicant) must use organisms from the same culture. They must be handled in the same way and delivered to the test containers at the same time.

6.0 TEST ORGANISMS

6.1 Laboratory Acclimation

Order amphipods to arrive between two and seven days before test initiation. Amphipods must be between 7 and 14 days old at test initiation. Place the amphipods in a culture tray containing well water at a temperature that varies by no more than 3°C from transport conditions. Acclimate the amphipods to test temperature. Hold amphipods at test temperature for 48 hours prior to initiating sediment testing. Remove any dead or moribund animals. Make sure water in the tray is constantly aerated. Check the amphipods daily, and monitor the health of amphipods by observing appearance. If more than 5% of the amphipods appear unhealthy during the 48 hours prior to the test, reschedule the test and immediately

arrange for another amphipod shipment. Renew the culture the day before the test with dilution water, and feed 10 ml YCT daily.

6.2 Amphipod Loading

Using a clean glass tube and bulb, transfer the amphipods from the culture tray into the test containers. Only transfer animals that are healthy and moving. Replace injured or stressed amphipods. Continue until each container has 10 animals. Maintain water temperature (23°C ± 1) by sorting animals in the constant temperature room where the test is being held.

7.0 MONITORING THE TOXICITY TEST

Measure temperature, dissolved oxygen, pH, conductivity, hardness, alkalinity and ammonia in the overlying water from each sediment sample at the beginning and end of the test. Also measure dissolved oxygen and temperature daily in each sediment test. Measure temperature, dissolved oxygen, pH, conductivity, hardness, and alkalinity at the beginning of each reference toxicant test, and temperature at the end of the reference toxicant test. Sample the test solutions as described below, and measure each parameter following the MPSL standard operating procedure for each measurement.

Temperature should be constantly monitored in reference toxicant and sediment tests.

On the first day of the test, before the amphipods are loaded into the test containers, use a clean graduated 10-ml pipette to remove a water sample from within 1 cm of the sediment surface for water quality measurement. Sample as close to the sediment as possible without disturbing the sediment or drawing fine particles into the pipette. Deliver the sample into water quality containers that are pre-labeled with the sample number. Use a clean pipette for each sample. Repeat this sampling procedure on the last day of the test, taking water quality samples from 1 cm above the sediment in the other water quality beaker.

Each sediment test container is fed 1 ml YCT per day. Reference toxicant tests are fed only on day 0 and day 2.

8.0 TERMINATING THE TOXICITY TEST

After 4 days of exposure, amphipod survival is determined in each of the reference toxicant tests, and final test temperature recorded. After 10 days of exposure, amphipods are removed from the sediment to determine rates of survival and growth. After sampling for water quality, carry test containers to the sieving table.

8.1 Preparation for Sieving

Before sieving, make sure the data sheet is ready on a clipboard, with a towel and pen next to it, in a place where it will be very difficult to go to the next beaker without remembering to write down your last count. Put on a lab coat, apron, boots, and gloves. Clear the water table of any old mud, if necessary, check water flows, and carefully examine your 400-µm screen to make sure there are no holes (>400µm).

8.2 Sieving, Recovering Amphipods, and Recording Data

Pour the entire contents of a test cone onto the sieve, hosing down the beaker walls to remove all particles larger than an amphipod. Spray water over the mud in the screen to wash particles away from the remaining amphipods. Break up any clods or mats, and continue spraying until all the fine sediment particles are removed. Quickly submerge the screen, catching the amphipods in the water surface tension. Use a screen to transfer all visible amphipods to a crystallizing dish then repeat the washing and submerging process to recover more amphipods. Continue this procedure until you are certain that you have recovered every amphipod. Count the number of live amphipods you have collected in the dish, and record that number immediately. Missing animals and animals that do not respond to a probe are considered dead. Once counted, pour the amphipods through a smaller sieve, and tweeze them off of the screen into dried, tared, numbered weigh boats. Carry the weigh boats, covered, to the drying oven and dry them overnight at 70ºC. Enter the number of amphipods in the weigh boat on the weight data sheet. Put empty beakers in a tray, and transfer them to the dish room, where they will be washed according to the glassware cleaning SOP.

The next day, weigh the dried weigh boats to the nearest 0.0000 g, and record the weights on the data sheet.

Take the completed data sheet to the office for data entry and analysis. Notify the data analyst that the data has arrived. Make sure the data sheets are placed in the proper location and that the person keeping track of the data knows where it is.

9.0 DATA HANDLING

Immediately after test termination, check the data sheet to determine whether home sediment controls have acceptable survival (mean of 80% or greater). If not, notify the project officer without delay.

Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Overlying water quality parameters must not vary by more than 50% during the test.

10.0 REFERENCES

Kemble NE, Brumbaugh WG, Brunson EL, Dwyer FJ, Ingersoll CG, Monda DP, Woodward DF. 1994. Toxicity of metal-contaminated sediments from the upper Clark Fork River, Montana, to aquatic invertebrates and fish in laboratory exposures. Environ Toxicol Chem 13: 1985-1997.

U.S. Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-99/064. Office of Research and Development. Washington, DC.

11.0 TEST SUMMARY

Species	*Hyalella azteca*
Test Duration	10 days
Endpoint	survival and growth
Renewals	none
Organism Source:	Aquatic Biosystems (Fort Collins, CO)
Age of Test Organisms:	7-14 days
Acclimation:	48 hours
Test Temperature:	23° ± 1° C
Dilution/Overlying water	Granite Canyon well water
Light intensity:	ambient laboratory illumination (10-20 μE/m^2/s)
Photoperiod:	16 hour light: 8 hour dark
Aeration:	Constant, 1-2 bubbles per second
Replication	8 (samples), 3 (reference toxicant)
Test Containers	1-L Imhoff settling cones (sediment), 250-ml plastic beakers (reference toxicant)
Test Volume:	15 ml sediment topped to 1 L with dilution water (samples), 100 ml (reference toxicant)
Loading:	10 amphipods per container
Feeding:	1 ml YCT daily (samples) or on day 0 and 2 (reference toxicant)
Overlying Water Quality:	pH, dissolved oxygen, temperature, conductivity, NH_3 alkalinity, hardness, (beginning, end), dissolved oxygen, temperature (daily)
Reference Toxicant	cadmium chloride ($CdCl_2$)
Daily Monitoring:	dissolved oxygen, temperature, feeding
Acceptability Criteria:	mean survival in dirt controls at least 80%

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